Studies on Insect Spermatogenesis.

V. On the Formation of the Sperm in Lepidoptera.

By

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With Plates 24–26.

In a paper published several years ago by Gatenby (1917 *a*) an account of sperm formation in Lepidoptera was given, certain features of which departed rather widely from the results of previous workers on similar material. Particularly interesting from my own point of view were the descriptions of the origin of the acrosome and the history of the mitochondria ('macromitosome' or nebenkern) in the spermatid, these matters having proved especially difficult to elucidate in the Hemiptera upon which I had resumed work early in 1919. As this work progressed, it became increasingly evident that the facts in the Hemiptera (and in other forms which I have studied subsequently) did not agree with certain important features of Gatenby's account, and aroused the suspicion that perhaps the facts in the Lepidoptera might be open to a somewhat different interpretation. I decided accordingly to put up some lepidopteran material for purposes of comparison, and during the last three years this has been done whenever opportunity offered. Considerable difficulty was encountered, due to the impossibility of accurately determining the age of pupae from their external appearance, and a proper range of preparations was therefore not easy to obtain. Certain stages are in fact still incomplete, but the general features are now

sufficiently clear to permit comparisons with other insects and it did not seem worth while to pursue the matter further at present. This paper will deal, therefore, only with the mitochondria and Golgi apparatus ('acroblasts'), and with them particularly in the older spermatids.

I must confess that when I first saw Gatenby's figures they aroused considerable scepticism. However, having now examined the material for myself, I find that we are actually in close agreement as to general appearances (so far as Gatenby's account extends), but that my interpretation differs materially from his in regard to several important points.

Material and Methods.

For material I have made use particularly of moths belonging to the family Saturniidae, the cocoons of which were readily collected during the winter and early spring in various localities adjacent to New York City. Of these moths this paper deals almost exclusively with Callosamia promethea. The cells in this form are rather small, but this disadvantage is offset to some extent by the large size of the testes. Callosamia (and other saturnids) has been studied by Cook (1910), to whose account reference may be made for the structural features of the testes, &c.[1] For purposes of comparison a study was also made of Pygaera bucephala, the form upon which the original work of Platner (1889) and the later classical studies of Meves (1900 and 1903) were based. Material was obtained from Mr. L. W. Newman of Bexley, Kent, Pygaera not being native to the United States.

Various methods of fixation were tried, but I found, in agreement with Gatenby, that the best results were obtained with Champy or Flemming without acetic acid (diluted in both cases with water). Gatenby is undoubtedly correct in his insistence on the necessity of eliminating acetic acid in the case of lepidopteran material, for even in its absence the mitochondria in the spermatocytes are very difficult to fix

[1] See also Dederer (1907) on the closely related form, Philosamia cynthia.

properly. The F.w.a. mixture gave the best general results and was particularly valuable for studying the Golgi bodies and acrosome: while for the mitochondria in spermatocyte and early spermatid stages only Champy was satisfactory. Fe-haematoxylin, sometimes with light green as a counter-stain, was employed exclusively for staining.

The Phenomenon of Polymegaly in Lepidoptera.

In his first paper on Pygaera, Meves (1900) called attention to the fact that the spermatocytes are of two sizes, the larger of which produces normal spermatids and sperms, while the smaller undergoes abnormal maturation divisions and produces abnormal (apyrene) sperms. The problem stated in this simple form by Meves has been complicated by the account of Munson (1906), who finds in Papilio two sizes of spermatocytes and spermatids both of which develop in a perfectly normal way. He believes that the small generation is to be considered 'normal' in size, since the large generation only makes its appearance late in the life of the butterfly. Munson unfortunately mixed up normal and abnormal stages in his account of sperm formation, and his statements are accordingly difficult to evaluate. Without at first recalling either of these cases, I noted independently that the spermatids in Callosamia are of at least two (possibly more) well-marked sizes. Of these, the larger generation certainly gives rise to normal sperms. On the other hand, the smaller spermatids apparently give rise as a rule to abnormal sperms, but nevertheless they are often found in an advanced stage of normal sperm formation, and considered separately would certainly not be thought abnormal. However, Gatenby (1917 *b*) states that the abnormal condition which results in the formation of apyrene sperms may exert its influence at different times, and it seems probable, therefore, that these small spermatids in Callosamia would undergo degeneration at a later stage: and cysts of small spermatids in later stages have in fact been found in course of changes possibly degenerative in nature. This being the case, my results would coincide

in a general way with those of Meves. The chromosome numbers in the large and small generations seem not to have been examined carefully, but if they are the same, as seems probable, the Lepidoptera might be considered as another example of the 'polymegaly' which Montgomery (1910) first described fully in Hemiptera.

I have recently (Bowen, 1922 *c*) given a full account of this particular type of spermatic polymorphism in the family Pentatomidae (Hemiptera), to which reference may be made for the details of this phenomenon. It may be pointed out, however, that in one important respect the conditions in the Hemiptera and Lepidoptera differ markedly; for while in the former all the cells, regardless of size, give rise to normally formed sperms, in the latter the small generation seems to give rise only to abnormal sperms, although the small spermatids may first proceed for some time on an apparently normal course, as noted above. Furthermore, in the Hemiptera all the spermatocytes and spermatids in a given testicular lobe are involved, while in the Lepidoptera only part of the cysts in each lobe are affected and these apparently without any noticeable plan. It may also be noted that in the Lepidoptera the appearance of the polymorphic cells seems to be an accompaniment of testicular old age, while such a relation is entirely lacking in the Hemiptera.

Notes on the Spermatocytes and the Spermatocyte Divisions.

The notes in this section deal only with Callosamia.

In Callosamia the mitochondria are present in spermatocytes of the growth period in the form of very numerous vesicular spheres or ovoids (fig. 42), approaching most nearly in general appearance those figured by Gatenby (1917 *a*) in Smerinthus populi and Pieris brassicae. They tend to be accumulated particularly in one region of the cytoplasm, and in my preparations are usually so closely packed that they give the impression of a mass of soap bubbles. I wish

especially to corroborate Gatenby's statement [1] of the duplex structure of these mitochondrial spheres, since upon this point depends a proper understanding of the formation of the nebenkern. Each sphere consists primarily of a droplet of some substance which has little or no affinity for the usual stains, and to which the name of chromophobic material has been appropriately applied. This material is enclosed in a delicate envelope of some substance which takes haematoxylin rather sharply, and is accordingly termed the chromophilic substance. I wish to call special attention to the fact that in a surface view of one of these mitochondrial vesicles this chromophilic layer is so delicate that as a rule it does not appreciably affect the transparency of the vesicle as a whole. Only around the periphery of the sphere, where the thickness of chromophilic material is sensibly increased by the effects of curvature, does it become clearly visible. In other words, a single (or double) thickness of chromophilic material would not be noticeable in a properly differentiated preparation. This point should be clearly understood, since upon the optical principle involved depends a proper interpretation of the 'spireme' in the nebenkern.

In the maturation divisions the mitochondrial vesicles seem rarely to retain their spherical shape, but, as Gatenby has also noted, are usually more or less drawn out in a direction parallel to the long axis of the spindle. This is sometimes so pronounced that in a cross-section through the region of the spindle pôles the vesicles, closely packed and decidedly elongate, are seen to radiate outward from the neighbourhood of the centrioles, reminding one very strongly of the conditions which I have described in the Hemiptera (Bowen, 1920). Something of this same appearance is shown by Gatenby (1917 *a*) in his fig. 48, which is especially interesting because the nucleus is still in the prophase, with its membrane intact. As the groups of daughter chromosomes separate during the anaphase, the mitochondrial vesicles become drawn out along the spindle,

[1] This structure of the mitochondrial vesicles was first correctly described by Meves (1900).

and are finally separated into two equal masses by the constriction of the cell wall. The vesicles then draw away from the region of the mid-body and, gradually rounding up, regain their former shape. This is shown particularly in the second maturation division, at the close of which the nebenkern is constructed (fig. 43).

The 'acroblasts' of Gatenby are obviously the representatives of the Golgi apparatus, as he has also indicated in a later publication. I wish only to call attention to the intimate structure of the individual Golgi bodies, since on this point I am not entirely in agreement with Gatenby's account. According to my observations each Golgi body is made up on a plan essentially similar to that which I have described in Hemiptera (Bowen, 1920), except that they are somewhat smaller, and hence less easy to analyse. Each Golgi rodlet is accompanied by a small, plate-like mass of material, which stains relatively less than the Golgi substance itself, and which, as I have shown elsewhere, is to be looked upon as a portion of the fragmented idiosome. The vesicular portion which Gatenby sometimes finds is obviously the equivalent of this idiosomic portion of each Golgi body; but I have never happened to see in my preparations any case in which it presented such a vesicular appearance.

In the maturation divisions the collection of the Golgi material around the spindle poles has been correctly described by Gatenby, but I cannot at present corroborate his statement that the 'acroblasts' are sorted out entire. The possibility of their undergoing more or less fragmentation, such as seems to occur so extensively in the Hemiptera (Bowen, 1920), ought, I think, to receive much more thorough study before a final decision is reached. In any event we seem to be agreed that the Golgi bodies are ultimately present in the spermatids in substantially the same form as in the spermatocytes.

Concerning the centrioles I have nothing new to contribute, except that Cook's (1910) statement concerning the loss of the tail filament in the first spermatocyte division of Callosamia is apparently incorrect, and was based in all probability on faulty technique.

The Formation of the Sperm.

The Structure and Fate of the Mitochondrial Body or Nebenkern.

The condensation of the mitochondrial vesicles to form the nebenkern (macromitosome of Gatenby) in Callosamia follows immediately upon the completion of the second spermatocyte division (fig. 43). For a detailed study of the method of this condensation the Lepidoptera offer the best material which has yet been found, the individual chondriosomes being so large and their constituent parts so clearly differentiated that the progress of events is not obscured by the stain, as in the case of the Hemiptera (cf. Bowen, 1922*b*). Gatenby (1917*a*) has given a series of figures showing the various steps in the process, and up to the stage shown in his figs. 15 and 38 the appearances in Callosamia are so nearly identical that additional figures seem unnecessary. According to this worker the process of condensation consists of a flowing together of the mitochondrial bodies, 'forming at first elongated structures, then loops, and finally filaments, the latter joining up gradually to form a tangled anastomosing figure', and finally, 'a perfectly coiled spireme'. With this interpretation of the process of condensation I am inclined to disagree, and would like to suggest an alternative explanation which, I believe, is also more in harmony with the later condition of the nebenkern.

I agree with Gatenby that the essential feature in the condensation phenomena is the flowing together or fusion of the mitochondrial vesicles. But, as I interpret it, this results not in forming loops or threads but merely larger aggregates of chromophobic material, the chromophilic material running together to form more or less complete partitions between the chromophobic droplets. Simultaneously the chromophilic material is withdrawn from the periphery of the mass as a whole, so that finally a spheroid of chromophobic material remains, subdivided in an irregular manner by chromophilic partitions. One might, indeed, liken the whole nebenkern to

a mass of soap bubbles. Although Gatenby's figures do not show the point satisfactorily, I have found that in Callosamia the surface of the nebenkern is often deeply indented at the points where the chromophilic partitions reach its periphery, emphasizing the impression of a vesicular mass. Doncaster and Cannon (1920) also show this very clearly in the nebenkern of the louse (see their figs. 18 and 19).

In contrasting these two interpretations it must be frankly admitted that the appearance of a thread-work is exceedingly deceptive. Indeed, it is possible that neither view can be conclusively proved without taking into consideration the later stages, in which the facts are very clear. Nevertheless two points against the thread-work interpretation may be urged. In the first place, in none of Gatenby's figures or in my own preparation is there any indication of the cut ends of a thread such as are obvious in sections of the chromatin spireme of dividing nuclei. It is exceedingly difficult to understand how such loose ends could be constantly avoided in sections. If, however, the structure of the chromophilic substance is that of a plate-work, the absence of ends is easily explicable. A second objection is based on the optical arrangements upon which the demonstration of the chromophilic substance in the spermatocyte chondriosomes was shown to depend (see preceding section). It will be clear from a consideration of these conditions that the visibility of the chromophilic material in the nebenkern may well depend on its disposition between closely adjacent chromophobic masses, the chromophilic septa being visible when seen on surfaces of sharp curvature, but invisible when seen in plane view, just as in the case of the individual chondriosome vesicles in the spermatocytes. Indeed, every feature of the condensation process becomes readily explicable if we think of it merely as a reduction in the number and arrangement of droplets of chromophobic material by the concentration of their chromophilic envelopes into more extensive separating membranes. Such a conception also helps us very much to understand the nature of the same process in other insects—the Hemiptera.

for example—in which the ultimate structure is clearly a combination of plate-work and vesicles, the intermediate steps being obscured by the less favourable structural features of the chondriosomes.

The next step in the condensation of the nebenkern is the withdrawal of the chromophilic substance from all contact with the periphery of the chromophobic mass as a whole, resulting in the formation of a clear zone enclosing the now centrally located chromophilic substance. This condition was described by Platner (1889), and will be recognized as a constant feature of the nebenkern in insects of all kinds. In accordance with the view elsewhere developed (Bowen, 1922 *b*), that all this early activity in the nebenkern is merely indicative of a centripetal condensation of the chromophilic material, I would interpret the complete withdrawal of the chromophilic material from the outer boundary of the nebenkern as merely the last step in the progressive withdrawal of this material, first from the outer periphery of the chromophobic mass as a whole, and then from the connecting pathways which at first traverse the outer chromophobic zone.

The stages in this process of withdrawal have been omitted by Gatenby so far as I can judge from his figures, and in my own preparations I have been unable to get completely satisfactory illustrative material. The process is undoubtedly difficult of analysis because it is during this interval that the rearrangements are completed which lead to the final organization of the chromophilic material into a more regular plate-work. A frequent appearance of this stage has been figured by Meves (1900, fig. 67) in *Pygaera*, and is shown still more clearly by Doncaster and Cannon (1920, figs. 20 and 21) in the louse. The central area of the nebenkern tends to stain more or less completely (as a result of slight imperfections in technique), concealing the detailed arrangements of the chromophilic material, while from this central accumulation delicate connexions pass out to the periphery of the nebenkern. These connexions are gradually withdrawn and the disposition of the chromophilic material now becomes progressively clearer.

In the larger spermatids of Callosamia, the general appearance is still exceedingly complex, perhaps justifying the representation which Gatenby gives in his figs. 40 and 42, for example. But in the smaller spermatids, where the chromophilic material is much less extensive, the condensation early reaches a point where arrangements are sufficiently simple for a practically complete analysis. Such a small spermatid of Callosamia is shown in fig. 44. The chromophilic material occupies the central area of the nebenkern, enclosed in a cortical zone of chromophobic substance, and arranged in the familiar 'onion' pattern which has been repeatedly figured by many workers on insect sperm formation. (Compare my figures from the Hemiptera (Bowen, 1922*b*).)

The further condensation of the chromophilic material now goes on rapidly, in a manner very similar to that which I have described in Hemiptera. In the Lepidoptera, however, the nebenkern begins to elongate soon after the cortical chromophobic area is established, this area remaining, in the immediately subsequent stages, as a characteristic feature of the nebenkern structure. With the elongation of the nebenkern the condensation of the chromophilic material has soon progressed to a point where the details of its arrangement become sufficiently simplified for satisfactory analysis. I have studied these later stages in both Callosamia and Pygaera, the latter being particularly good on account of the large size of the spermatids. An early stage in the elongation of the nebenkern of Pygaera is shown in fig. 1, and in fig. 3 a cross-section through a nebenkern of the same stage. Figs. 4 and 5 are similar views at a slightly later stage in the elongation, and fig. 6 is a cross-section of the nebenkern in a still older spermatid. In fig. 45 is shown a cross-section of a nebenkern in Callosamia when its elongation is well begun, and fig. 46 is a total view of a spermatid at a stage intermediate between that of figs. 4 and 8. Figs. 8, 10, 14, and 13 are progressively later steps in the condensation of the chromophilic substance.

A comparative study of this series of longitudinal and cross-

sections will, I think, make clear the nature of the processes at work, and their extraordinary similarity to the conditions which I have described fully in the hemipteran nebenkern. In the first place, it is abundantly clear that a 'spireme' is in these stages an impossible interpretation. There is no conceivable arrangement of a thread in the nebenkern which will produce a regular bounding line in both long and cross-sections of the chromophilic material. Such an appearance can only be produced by a continuous surface, which, in accordance with the optical principles previously referred to, would, if of proper thickness, produce the effect of a simple line or thread when seen in optical section. In other words the chromophilic substance is arranged in a plate-work, exactly as it is in the Hemiptera. This is further proved by the fact that the chromophilic material now stains with sufficient intensity to be visible in surface views (fig. 4, for example), a result impossible with an open thread formation. The cross-sections particularly show that this plate-work is arranged as a series of concentric shells in which, however, more or less extensive irregularities occur. The longitudinal sections are not so satisfactory, since the section is rarely exactly parallel to the long axis of the nebenkern, and this, coupled with the irregularities in the plate-work and the difficulty of differentiating the various layers with equal clearness, makes the picture particularly confusing in the earlier phases of elongation.

This series of figures shows further that the chromophilic substance is constantly diminishing in volume, with an increasing simplification of its structural arrangements. Indeed, in the later stages of condensation the cross-sections are especially simple (figs. 47 A and 9 A and B), and exhibit in every particular an exact parallelism with the same condensation steps in Hemiptera. The final result of this process of condensation is the complete disappearance of the chromophilic material, the last stages in this process being shown in figs. 8, 14, and 13, the last two being from the same cyst. The ultimate fate of the chromophilic substance, and, indeed, all of the stages which directly precede its complete disappearance are thus exactly

comparable to those which I have described in Brochymena (Bowen, 1922 *b*). These later stages seem for the most part to have been overlooked by Gatenby—at all events his account of the fate of the chromophilic substance (' spireme ') seems to be entirely incorrect. The source of his statement as to the breaking up of the ' spireme ', as shown in his Text-fig. 3, will be considered in a later paragraph.

It will be convenient at this point to refer briefly to the parallel course of events in the chromophobic material. As the nebenkern draws out along the axial filament of the tail, both chromophilic and chromophobic substances are at first involved (fig. 1). Very soon, however, the chromophilic substance ceases to elongate (figs. 4 and 46), and begins gradually to shorten up as its dissolution advances. The chromophobic material, on the other hand, continues to elongate very rapidly (fig. 8), and tends gradually to become spun out towards both ends with a median swelling in the region occupied by the remains of the chromophilic plate-work (fig. 46). The proximal end of the nebenkern (not to be made out in fig. 46) seems to be anchored in the vicinity of the insertion of the tail filament, as is the case in other insect sperms. The continued spinning out of the nebenkern results in the production of a mitochondrial sheath for the tail filament, exactly as in the Hemiptera.

It has long been known that the nebenkern becomes divided into two equal masses in many insect spermatids (Hemiptera and Orthoptera), prior to the spinning-out process, while in Lepidoptera, according to the current descriptions, this division is entirely omitted. In my study of the nebenkern in Hemiptera (Bowen, 1922 *b*), I noted for the first time the relation between the final disappearance of the chromophilic matter and the complete division of the nebenkern into two equal parts. This relation was found to hold true in the Orthoptera and Coleoptera also (Bowen, 1922 *d*), and I ventured the guess that in the Lepidoptera, ' a division of the nebenkern will be found to occur once the chromophilic substance has been disposed of ' (Bowen, 1922 *b*, p. 69). This point has been

very carefully examined, especially in serial cross-sections of the nebenkern, and it is now clear that this guess was correct in every particular. As in the Hemiptera the division of the nebenkern is foreshadowed by the symmetrical disposition of the chromophilic material (figs. 3, 5, and 45), and the division itself is accomplished in the regions unoccupied by chromophilic material soon after elongation begins. In the region of the chromophilic material itself, however, the division is not (usually) completed until after the final act of dissolution, a point in which the Lepidoptera agree with the Coleoptera in which the splitting of the nebenkern is delayed in a somewhat similar manner. In the Lepidoptera, however, there seem often to be more or less local irregularities in the division process, and it thus happens not infrequently that the final remnant of the chromophilic material is left to complete its dissolution in one of the nebenkern halves, while the division plane is completed (fig. 9 B). The general features of the division process as outlined above are well shown in figs. 9, 12, and 47. In fig. 9, which represents a nebenkern at the stage of fig. 8, the division above (and below) the chromophilic substance is completed (fig. 9 C), but in the region of the plate-work it is still incomplete (fig. 9 A), with the exception of cases like fig. 9 B already noted. In fig. 47 cross-sections of a nebenkern like fig. 46 are shown. The more spun-out portions at the ends of the nebenkern masses are shown in fig. 47 C, while the parts nearer the middle are shown in fig. 47 B, and the region of the plate-work itself in fig. 47 A. Comparison of figs. 3, 5, and 6, with figs. 9 A and B, and fig. 45 with fig. 47 A, shows clearly how the structure of the plate-work becomes progressively simplified as the chromophilic material condenses. Just before its final dissolution the plate-work is reduced to a simple ovoid shell (figs. 9 A and 14), such as I have described in Hemiptera and Coleoptera. (Compare also the figures of Doncaster and Cannon (1920) in the louse.) In these late condensation stages the plate-work, as against the thread-like structure of the chromophilic material, seems to me unquestionable. As the figures show, the axial filament lies in the

groove between the two halves of the divided nebenkern, just as in other insects.

After the division of the nebenkern (probably) the chromophobic material begins to develop constrictions, at first in the more distal region of the sheaths, which divide it into a series of bead-like masses. This process is shown particularly well in figs. 7 and 10, although the development of these bleb-like swellings is often (usually ?) deferred until after the disappearance of the chromophilic material. The last-mentioned figure is from a cyst of abnormally large sperms, and this may account for the unusually early development of the swellings. These bead-like masses are rapidly separated from each other by the spinning out of the intervening chromophobic material. As a rule these delicate connecting strands are not well seen, and the tails look like a series of clear vesicles often without any apparent connectives (fig. 11). This is particularly true in the later stages of sperm formation, when the vesicles seem merely to be scattered loose along the tail filament (figs. 17, 25, 35, 37, 40, and 52 for instance). However, in material fixed in Flemming without acetic acid and strongly stained in Fe-haematoxylin the chromophobic material can sometimes be coloured very sharply, and it is possible in favourable cysts to make out the actual structure of the nebenkern derivatives with the greatest clearness. From such preparations it is evident that the original halves of the nebenkern have become spun out into delicate threads which run parallel to the tail filament and at intervals bear the bleb-like swellings, now present in larger number but individually much reduced in size. The general appearance is exactly like that in Euschistus (see Bowen, 1922 *b*, fig. 27). It is clear that these swellings are homologous with the ' tail vesicles ', the formation of which was described fully in the Hemiptera (Bowen, 1922 *b*).

Finally, the central substance which I have described in detail in the Hemiptera (Bowen, 1922 *b*), and less completely in Orthoptera (Bowen, 1922 *d*), remains to be considered. As in the Hemiptera this material first becomes visible in the chromophobic area of the nebenkern during the middle

stages in the dissolution of the chromophilic substance (figs. 4, 5, and 6). It does not stain very sharply with Fe-haematoxylin and its exact morphology is difficult to make out. It seems, however, to consist of small droplets which tend to run together to form more or less irregular threads traversing the chromophobic material in a direction parallel to the long axis of the nebenkern (fig. 7, 8, and 13). As the chromophilic material disappears, the central substance becomes more conspicuous (fig. 10), and in cross-sections of the nebenkern appears exactly as it does in the Hemiptera (figs. 9 C and 12 B). In fig. 47 B it has become condensed into a single strand in each half of the nebenkern. (Compare with Holmgren's (1902) account in Silpha, fig. 9 M.) As in Ceuthophilus (Bowen, 1922*d*), the central substance is present in the tail vesicles in much the same form in which it appears in the unconstricted nebenkern (figs. 10, 11, and 48). A more detailed account of the central substance may be omitted here, since I have discussed the subject in another paper (Bowen, 1922*b*), to which the reader is referred for comparative details, especially in the Hemiptera.

It will be observed that Gatenby has failed to recognize the central substance in the lepidopteran nebenkern. In studying his figures I have come to the conclusion that the thread-like formations shown in the nebenkern in his figs. 47 and 20 are to be interpreted as central substance. He describes these threads as resulting from the breakdown of the 'spireme', a conclusion which he seems to have reached on the basis of the supposed structure of the early nebenkern, without having traced out the necessary connecting links between the two. My observations leave no doubt that the chromophilic and central substances are morphologically distinct, and that whatever the structure of the chromophilic material may be, that of the central substance is in no way dependent upon it.

So far as my observations go they indicate that the threads, spun out from the halves of the nebenkern, ultimately form a sheath for the tail filament of the mature sperm, as described in other insects by various workers. The fate of the tail vesicles is not known.

Notes on the Centrioles and the Transformations of the Spermatid Nucleus.

I have found the centrioles in the lepidopteran spermatid exceedingly difficult to demonstrate with any degree of satisfaction. I am, therefore, unable either to confirm or deny the extraordinary account given by Gatenby. His statement that one of the centrioles is cast off ought certainly to receive the most careful examination. It is usually an easy matter in the insect spermatid to demonstrate the centrioles in some form or other at the point of insertion of the tail filament, but in the moths which I have studied even this has usually proved impossible. Figs. 28–30 show at the end of the axial filament a small granule, which is presumably the centriole(s), and Gatenby's fig. 51 seems to show something similar. I wish only to point out here that in the Lepidoptera, as noted by many workers, the head of the spermatid is bent very sharply at the point of insertion of the axial filament, so that the original insertion seems to be near the anterior side of the nucleus rather than at its base, as is customary. Subsequent stages indicate that this may actually be the case, the centriole perhaps shifting its position to the base of the nucleus when the latter elongates to form the sperm head.

The breaking up of the chromosomes at the close of the second maturation division offers no points of special interest. The chromatic material becomes eventually spread out in a thin and slightly uneven layer on the inner wall of the nucleus (figs. 1, 4, 8, and 13), somewhat as in the Hemiptera (Bowen, 1922 *a*). During the later stages in the spinning out of the nebenkern halves, a rearrangement of the chromatic material is accomplished. This results in the appearances shown in figs. 15 and 16, in which one gets the impression that a portion of the nucleus is being cleared up by the withdrawal of the chromatin. This seems actually to be the nature of the process, for subsequently the nucleus appears divided rather sharply into two areas, one of which is perfectly clear and transparent, while the other retains the chromatic material probably still

in the form of a thin layer on the nuclear wall (figs. 18 to 20). The exact appearance depends, of course, on the orientation of the nucleus with respect to the observer. This rearrangement of the chromatin is again reminiscent of the hemipteran spermatid (Bowen, 1922 *a*), with the difference that in the Lepidoptera the clear area seems to be opposite the insertion of the tail filament, rather than around it. as in Hemiptera. This arrangement of the chromatin is very clear in Pygaera, but made out with great difficulty, if at all, in my preparations of Callosamia. Only in rare cases did the chromatin stain with characteristic intensity in any of my preparations, the fixation in Flemming without acetic acid being apparently responsible for this. I have noted the same result in the testes of other animals. It is an exceedingly fortunate failure, for it allows of many observations which could not possibly be made if the sperm head were intensely coloured. Occasionally, especially in later stages, some of the heads in a cyst will stain intensely (compare figs. 40, 41, and 60), a result which makes easy the determination of the exact limits of the head itself.

The division of the head into the staining and non-staining areas noted above, seems to have been made out by Platner (1889), but his figures do not give a very adequate idea of the actual conditions. During the early stages in the elongation of the acrosome, the clear area gradually disappears (figs. 21, 22, and 26), and the head then stains uniformly (figs. 28, 29, &c.). During these latter changes the head seems to undergo a diminution in size, a phenomenon which is met with not uncommonly (always ?) in insect sperm formation. The nucleus, at first spherical, gradually elongates (figs. 35 to 41 and 57 to 60), as in other insect spermatids, and eventually becomes a long, delicate rod, not unlike the sperm head in Hemiptera (Bowen, 1922 *a*).

Aside from the differentiations already noted in the spermatid nucleus, I have also constantly found within it a small darkly-stained body, of spherical shape, which is perhaps of nucleolar origin, possibly related to the intra-nuclear body which I have described in the hemipteran spermatid (Bowen, 1922 *a*). In

Callosamia (where it was observed by Cook (1910)) this body seems to appear very early (fig. 44), but in Pygaera it becomes conspicuous only in later stages. In the latter it can be recognized as a minute granule at the time when the elongation of the nebenkern is well started (figs. 4, 8, and 13), and subsequently (figs. 15 to 18) it becomes larger and much more prominent. In Callosamia it may divide into two parts (often unequal) at a fairly early period (fig. 46). In Pygaera the division is delayed until the clear area (in which it tends to be located (figs. 20 and 22)) in the head is differentiated, and when it does occur, it tends to take place in all the heads of a cyst (figs. 19 and 20). In later stages this body seems to become less conspicuous (figs. 31 and 35), and, I believe, eventually disappears entirely, being presumably dissolved in the nuclear sap. Not infrequently this body is in line with the tail filament, and it might easily be mistaken for a centriole (figs. 52 and 57). As far as I can make out, however, it has no real connexion with any extra-nuclear structure.

Finally, I would like to mention in passing a phenomenon which seems to have been overlooked by previous workers on Lepidoptera, and which I myself do not fully understand. An examination of cysts of sperms in later stages of transformation (figs. 36 and 58, for example) shows the elongated acrosomes to be embedded in a mass of large, clear vacuoles which have the appearance of a large number of soap bubbles crowded together. I supposed at first that these vacuoles represented an elaboration of the protoplasm of the so-called nurse-cell in which the sperm heads of insects are characteristically embedded. Further study indicated that this view was not tenable, for at a slightly earlier stage more or less separated vacuoles could be found among the heads without any apparent connexion with cells of the cyst wall. I would like to suggest, as a possible explanation of their origin, that these vacuoles represent material extruded from the nucleus probably at the time of its diminution in size, and comparable to the similar extrusions which seem to occur in the Hemiptera (see Bowen, 1922*a*) and other animals. This view is borne out by the

fact that occasionally (in Callosamia) cysts are found at about the age of fig. 56 (or later), in which each sperm head is enclosed in a clear vacuole—presumably the vacuoles noted above which have perhaps failed to be formed in a normal manner. Something of this appearance is shown by Cook (1910) in her figs. 132 and 134 of Automeris. This opinion is further strengthened by the fact that in degenerate (apyrene) sperms of Callosamia I have found that each head (now moving back in the tail region) is accompanied by a droplet of non-staining material (see Munson, 1906, fig. 49), over which the acrosome passes. If these vacuoles are a product of the nucleus we should expect just such a disposition of them in the apyrene sperms; but their connexion with the nucleus is not easily accounted for on any other explanation of their origin. In normal cysts, as the sperms grow older, these vacuoles seem gradually to disappear, but their exact fate has not been traced.

The Golgi Apparatus and Acrosome.

My chief interest in examining spermiogenesis in Lepidoptera was centred on the origin and development of the acrosome, especially in view of the account given by Gatenby (1917 *a*) of the rôle of the Golgi bodies (his acroblasts) in this process. According to Gatenby, all the Golgi bodies become swollen into vesicular spheres during the early spermatid stages, and these spheres fuse to form the basis of the acrosome. In each of these spheres there is differentiated a small, darkly-staining granule, which is also involved in the construction of the acrosome itself. In a subsequent statement Gatenby and Woodger (1921) say, 'Our recent observations . . . on several other moths (e.g. Biston) have shown that in these insects much of the apparatus finally passes as isolated crescents, spheres, or dictyosomes into the elongating tails of the spermatozoa.' The problem is thus left in a very unsettled condition. In my previous papers on spermatogenesis I have endeavoured to show that the acrosome is a product of the Golgi apparatus plus idiosome, but that neither of the latter structures is made

d i r e c t l y into the acrosome. After the acrosome is formed the Golgi complex as a whole is cast off and has no further connexion of any kind with the acrosome. I have recently developed my views on this subject in a more general form (Bowen, 1922 *e*), and it will be the purpose of this section to show how the observations of Gatenby can be harmonized with my previous results.

The early steps in the formation of the acrosome cannot be analysed with any satisfaction in Callosamia on account of the very small size of the acrosome in this form. In Pygaera my material begins at a point where the acrosome is already nearing completion. I will accordingly refer to Gatenby's figures for the earliest stages, the essential features of which are also shown clearly in the older spermatids of my Pygaera preparations.

My observations confirm the statement of Gatenby and Woodger (1921) concerning the casting off of the Golgi bodies, but I would go further and state that not merely ' much ', but all of the Golgi apparatus is thus disposed of. The Golgi bodies can be seen in any of the older spermatids at varying distances from the nucleus (figs. 8, 10, 13, 46, and 48), and in much later stages they can be found scattered in groups at various points along the sperm tail. Furthermore, they show no evidence of a vesicular structure, but they do show, in favourable cases, the differentiation into Golgi rodlet and idiosomic substance which I have found to be so characteristic of them in the primary spermatocyte. How then does the acrosome arise? Gatenby's figs. 36 and 37 indicate, I believe, the essential features of the answer to this question. In these figures each of the acrosomic vesicles has attached to one side a Golgi rodlet, the idiosomic substance not being shown. I have been similarly unable to make out the idiosomic material in Pygaera, but the conditions in Callosamia leave no doubt that it is present, but temporarily obscured, after ordinary staining, by the development of the vesicles. In other words I would interpret the vesicles as differentiation—rather than direct transformation—products of

the Golgi bodies. These spherical vesicles are then deposited on the nuclear wall, and gradually fuse to form the acrosome, the granules differentiated within them fusing at the same time to form a single large acrosomal granule. As the acrosomal vesicles are deposited the Golgi bodies are cast off, as in the formation of the acrosome in other animals, and move off down the tail. It is clear in my Pygaera preparations that the formation of the vesicles by the Golgi bodies is not completed simultaneously in all of them, but rather that there is a gradual production and deposition of the vesicles extending over a considerable period, and concluded only at a relatively late stage (figs. 13, 15, 16). Figs. 1 and 2 show the latter part of the acrosomal formation in progress. The acrosomal granule in Pygaera stains very intensely and is of extraordinary size, often concealing the vesicular portion, especially if the latter is not well differentiated by the staining. Several Golgi bodies are grouped around the acrosome, and particularly in fig. 2 one gets the impression that one or two of them are in the act of depositing the small acrosomal vesicle which each has elaborated. Not infrequently the acrosome is multiple, as in fig. 2, one portion being much the smaller, but later on these parts always merge into a single acrosome. Gatenby shows this process in his Text-fig. 4, and with the general plan of this figure I am in entire agreement. The Golgi bodies seem to clear away from the acrosome as they deposit their quotas, and thus they tend to be scattered along the tail rather than to be collected in a single group. In the later stages of deposition the acrosome in Pygaera can be very clearly separated into its two fundamental constituents—the intensely-stained acrosomal granule and the clear, unstained acrosomal vesicle (figs. 13 and 15).[1] The contour of the vesicular portion is at first rather irregular, which I take to be indicative of its multiple origin; but the irregularities are gradually

[1] I have found the material of the acrosomal vesicle very difficult to differentiate sharply in the earlier stages. It is, furthermore, often obscured by the enormous acrosomal granule, so that in my figures of young spermatids the vesicular part of the acrosome may not appear at all.

smoothed out (figs. 15 and 16), and all traces of its original composition are lost.

In Callosamia the whole acrosome tends to stain darkly during the period of formation, a phenomenon which I have also noted in Hemiptera and in Ceuthophilus when the staining is not perfect. This is presumably the source of the similar condition in Callosamia, for when the Golgi bodies have all cleared away the acrosome is clearly constructed on the same plan as in Pygaera. The difference in size is, however, astonishing, for in Callosamia the whole acrosome is exceedingly small and inconspicuous (figs. 50 and 51). Nevertheless, it is differentiated into a vesicular and a granular part exactly as in Pygaera. The granule tends to be slightly elongate rather than rounded in the stage at which I have first succeeded in differentiating it. Earlier stages (figs. 46 and 48) show very clearly the relation of the Golgi bodies to the forming acrosome, but I have not been able to make out their individual contributions, which are presumably very minute. Fig. 49 shows an interesting case in which the last Golgi body is just on the point of separating from the acrosome. In this case the Golgi body appears to be a fusion product of several smaller Golgi bodies.

The interpretation which has here been given furnishes, it seems to me, a complete explanation of Gatenby's results, and brings the lepidopteran acrosome into harmony with the conditions as we now know them to exist in many other animals. According to the idea which I have developed each Golgi body in a lepidopteran spermatid would be an acroblast on a small scale, and the formation of the acrosome is thus a multiple process. In its essential outlines it is, however, clearer than in the case of the grasshopper which I have described in another place (Bowen, 1922 *d*). For the relation of this type of acrosome formation to more familiar cases in other animals the reader is referred to my paper on the acrosome, in which I have tried to bring the whole series of known facts under a common view-point (Bowen, 1922 *e*).

Gatenby's account leaves the further history of the acrosome

practically untouched ; but as its later development offers a number of interesting features I have thought it worth while to work out the subsequent events in Callosamia and Pygaera from a comparative standpoint. The acrosome in Pygaera is particularly favourable for study because of its large size.

In Pygaera the acrosome when finally deposited consists, as noted above, of a clear vesicular portion, the acrosomal vesicle, and a very large, darkly-stained granule, the acrosomal granule. The latter is presumably contained within the former, but the large size of the granule gives one the impression rather of a bipartite mass (figs. 15, 16, and 17). The vesicular material seems now to undergo further concentration, its outline becoming very clearly marked. Meanwhile the granule becomes slightly drawn out into a spindle shape, with the vesicular material applied along one surface (figs. 18 and 19). In fig. 19 various aspects (oblique, cross, and longitudinal optical sections) of the acrosome at this stage are shown. It will be noted that the acrosome tends to be located on the nuclear membrane at the edge of the chromatic lining.

It soon becomes evident that the assumption of the spindle form by the acrosome is merely the initial stage in a process of elongation which now progresses rapidly (fig. 20 et seq.). In this elongation one end of the acrosome is temporarily fixed near the anterior pole of the sperm head, the acrosome thus growing backward over the nuclear wall until it projects considerably behind the nucleus (figs. 20, 22, 24 to 27). Having reached the stage shown in fig. 27 the acrosome becomes detached anteriorly, and slides bodily forward until the originally posterior free end becomes applied to the anterior nuclear wall. Steps in this remarkable migration of the acrosome are shown in figs. 27 to 31. Eventually the posterior tip of the acrosome seems to be attached to the nucleus at the point of insertion of the axial filament (figs. 31 et seq.). The cytoplasm in the head region is at first carried forward with the acrosome (figs. 28 to 33), but in later stages it gradually moves backward (figs. 35 to 38) until the entire head region

is free from cytoplasm (figs. 39 to 41), exactly as in other insect sperms.

After moving into its definitive position the acrosome continues to elongate, and eventually becomes spun out into a remarkably long, delicate apical piece (figs. 33 to 41). Fig. 41 is the latest stage in which I have seen the acrosome in anything like its entirety, but the head of the sperm itself is still in an intermediate stage of elongation. The extraordinary size relations of the acrosome in the mature sperms can be inferred from Meves' (1903) fig. 152. (See also his descriptive account.)

To go back now to the early elongation stages, the structural features of the acrosome itself deserve further attention. Once the acrosome has become markedly elongate I have found it as a rule impossible to differentiate the vesicular material which is obscured by the heavily-stained ' granule '. However, in cross-sections, especially of intermediate stages in elongation, the two materials can be readily distinguished (fig. 34), and there can be no doubt that the two original constituents of the acrosome remain distinct at least for a long period. A comparison of figs. 32 and 33, and 36 and 37, with fig. 34, suffices to explain the appearances presented by the acrosome when viewed from different aspects. It will be evident that in figs. 33, 37, and 38 the acrosome is turned so that the acrosomal granule is seen in plane view, while in figs. 32, 36, and 39 the acrosome is seen in what one might call a side view (compare fig. 34). As the figures show, the side of the acrosome on which the vesicular material is disposed is usually turned towards the major axis of the sperm.

Within the acrosome itself various changes take place, the first of which to be noted is the production, at the temporarily attached end, of a clear area, which seems to be the first part of the acrosome to free itself from the nuclear membrane prior to migration (figs. 26 to 30). This clear zone is subsequently lost, and another one develops at the permanently attached end of the acrosome, while at the very point of attachment a darkly-stained body appears (figs. 31 to 39). Whether this represents a differentiation of the acrosome,

or is possibly of centriolar origin, I have not been able to determine. At any rate this seems to be a common point of attachment for both acrosome and tail filament. There are indications that subsequently (fig. 40 and later) the centriolar apparatus, or a portion of it, becomes shifted to the base of the sperm head, but I have not found my material satisfactory for a detailed study of these phenomena.

In Callosamia the general progress of events is exactly parallel to that in Pygaera, with the possible exception of the method of orientation of the acrosome. The structure of the acrosome in this moth does not permit detailed study of a possible forward migration, and it is possible that the migration may occur in a different way. In the early figures, it will be seen that the acrosome is deposited at a point some distance removed from the insertion of the tail filament. As the acrosome begins to elongate, however, it apparently migrates anteriorly (fig. 52), and becomes attached by one end near the insertion point of the axial filament (figs. 52 et seq.).[1] From this point on the exact course of events is uncertain, and whether or not, with the elongation of the acrosome, there is a further change of orientation, as in Pygaera, has not been ascertained. However, appearances like that of fig. 54 suggests that perhaps the acrosome at first grows posteriorly, as in Pygaera, and shifts later into its definitive position. As already noted in Callosamia, the acrosome, as originally deposited, is relatively much smaller than in Pygaera (figs. 50 and 51), and we should therefore expect its later stages to be much more delicate in structure. As a matter of fact this expectation is exactly realized, and almost from the beginning of elongation the acrosome has a thread-like form, in which it is impossible to distinguish the two acrosomal constituents (figs. 51 to 59).

The various steps in the forward growth of the acrosome are shown clearly in figs. 56 to 59, and present no points of special interest. The length of the acrosome in the mature sperm as compared to the length of the sperm head is much less

[1] It is possible that a similar migration occurs in Pygaera, where it would be masked by the large size of the acrosome.

in Callosamia than in Pygaera, correlated apparently with the very much smaller amount of available acrosomal material. The thread-like nature of the acrosome and its attachment so near the insertion of the axial filament offer the possibility of a natural error in interpretation which should be guarded against in the study of other sperms. If one studied only the later stages of sperm formation, or observed the earlier ones inaccurately, a most obvious conclusion would be that the acrosome was really a forward growth from the spermatid centrioles, comparable in its method of formation to the tail filament. It is possible that some of the accounts which have been given of the rôle of centrioles in acrosome formation may actually be traceable to errors of this nature. In any event it is clear that great care should be exercised in the future in interpreting thread-like formations in the sperm head, since it is now evident that topographical relationship to the centrioles may not be in the least degree indicative of organic relationship. It is interesting to note in this place that Goldsmith (1919) has described a thread-like formation in the sperm head of Cicindela, without, however, offering any explanation of its homologies. I think it probable that the facts made out in Callosamia will furnish a clue to the enigmatic structures described by Goldsmith.

The Apyrene Sperms.

In the saturnid moths large numbers of apyrene sperms are formed after the normal sperm formation has been largely completed. The nucleus in every case is reorganized in the spermatid in a normal manner, as Gatenby (1917*b*) found in Pieris brassicae. However, at an early stage the spermatid becomes visibly abnormal by reason of the improper orientation of the nucleus and nebenkern, their relative positions being exactly reversed in the abnormal spermatids. The result is quite striking when an entire cyst is observed, the nebenkerns being adjacent to the cyst wall instead of the nuclei, as is so characteristic in insect testes.

As the nebenkern elongates the nucleus moves back along

the tail, as Gatenby shows for Pieris brassicae. The acrosome behaves at first in a normal manner, so far as can be judged from stages corresponding to that of fig. 55, for it can be readily found in the form of a delicate, elongate rod or thread attached to the nucleus. Gatenby (1917 *b*) is apparently dealing with an acrosome of this kind in Smerinthus populi, as indicated in his fig. 9, which shows a condition very similar to that in Callosamia. As I have shown, the early elongation of the acrosome is in no way dependent on the elongation of the nucleus, and Gatenby's conclusions (pp. 474–5 of his paper), from the figure mentioned above, seem to me entirely unwarranted. On the whole, the degeneration phenomena in the lepidopteran testis seem still to offer many problems which call particularly for an intensive study of the whole germ-cell cycle for their adequate solution.

Conclusion.

As I have indicated elsewhere the primary purpose of this study was to compare the fundamental differences in the sperm formation of Lepidoptera and Hemiptera as brought out by the work of Gatenby and myself. As a result of my observations on the Lepidoptera it appears that such differences as actually occur are primarily ones of detail, and that in all essential respects these two insect groups have a remarkably similar spermiogenesis. Indeed, a further comparison with the Orthoptera and Coleoptera which I have studied gives unmistakable indication of the fundamental similarity in the processes of sperm formation in all insects, and discounts in a most decided manner the bizarre accounts of insect spermiogenesis with which the older literature is full.

This seems to be particularly true of the nebenkern, the history of which I have treated in another paper (Bowen, 1922 *b*). In that paper I made extensive use of Gatenby's studies of the Lepidoptera because in this group conditions seem unusually favourable for an exact analysis of the early condensation of the nebenkern. I then accepted the account of the mitochondrial 'spireme', and suggested that the neben-

kern 'patterns' might follow either a 'spireme' type or a 'plate-work' type. As a result of the studies here recorded, however, I no longer find myself able to accept the reality of the 'spireme', and I now believe that the evidence is indicative of a plate-work in the lepidopteran nebenkern essentially like that which I found in the Pentatomidae. Indeed, it appears quite likely that the 'spiremes' sporadically figured by other workers and referred to in my previous paper are all to be accounted for on similar grounds and to be equally explicable on the basis of their plate-work structure. A plate-work seems to be the fundamental structure of the chromophilic substance in the nebenkerns of all insects.

In this connexion the results of Doncaster and Cannon (1920) on the louse are of special interest. These workers have employed modern technique, and have reached a conclusion which is in accord with my own interpretation of the vesicular nature of the early nebenkern. Their account is characterized by the remarkable conclusion that the nebenkern is formed and goes through the earlier condensation stages in the primary spermatocyte. In the single and abnormal maturation division it passes bodily into the functional spermatid, and is thus already well along in its evolution when sperm formation proper is just beginning.

Finally, one point in Platner's (1889) old description of Pygaera suggests interesting matter for comparison. I have long been puzzled by this author's small mitosome, which was thus designated because of its supposed relation to a part of the spindle fibres. It is clear now that what Platner really saw was the acrosomal granule, and his account of its elongation, occurring at first in a posterior direction, is remarkably suggestive of my own observations. It is evident from his figures, that the small mitosome was not intensely stained by his technique, and his failure to make out the final fate of the 'small mitosome' (acrosome) is probably to be ascribed to the increasing difficulty of demonstrating it as its form became less compact. Gatenby's revival of the term as the micromitosome, for a cytoplasmic granule somewhat

recalling the chromatoid body of other workers, seems thus to be a rather unfortunate one, as it is quite improbable that this body has anything whatever in common with the acrosome.

Summary.

1. The lepidopteran nebenkern passes through a series of condensation phenomena which are essentially similar to those previously described in Hemiptera.

2. The structure of the chromophilic material is probably that of a plate-work rather than a spireme.

3. The central substance is developed in the nebenkern exactly as in other insects.

4. The Golgi bodies in all probability give rise each to a small vesicle, these vesicles fusing gradually to form the acrosome.

5. As in other insects, the Golgi bodies are not directly transformed in the building up of the acrosome, but, after giving rise each to its miniature acrosomal vesicle, they pass back along the tail and are probably cast out of the sperm along with other detritus in the concluding stages of spermiogenesis.

6. The essential parallelism existing between the formation of the sperm in Lepidoptera and in other insects, especially the Hemiptera, is particularly emphasized.

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EXPLANATION OF PLATES 24–26.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. At so great an enlargement it has of course been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly, those of Pls. 24 and 25 to an enlargement of approximately 2,530 diameters, those of Pl. 26 to approximately 3,000 diameters. All of the figures are from material fixed in Flemming without acetic acid, except figs. 42, 43, and 44, which are from material fixed in Champy.

Reference Letters.

A, acrosome. *B*, nuclear body of doubtful nature. *C*, centriole(s). *f*, tail filament. *G*, Golgi apparatus = acroblasts (where the acroblasts are much scattered, only a few representative ones are specifically labelled). *K*, nucleus. *M*, mitochondria. *N*, nebenkern. *S*, central substance. *V*, vesicles developed on the mitochondrial tail sheaths.

Plate 24.

Explanation of Figures.

All the figures are from Pygaera bucephala. Figs. 1, 2, and 3 are from the same cyst of developing sperms; likewise figs. 4 and 5, and figs. 8 and 9; figs. 10, 11, and 12 are from a cyst of abnormally large spermatids.

Figs. 1, 4, 8, 10, and 13.—Progressive stages in the elongation of the nebenkern. The acrosomal granule is stained intensely black.

Fig. 2.—Section through a spermatid nucleus, showing the acrosomal granule in two parts, and numerous Golgi bodies, some of which seem to be engaged in depositing their quota of acrosomal material.

Figs. 3, 5, and 6.—Cross-sections through the nebenkern in successive stages of its elongation to show the structure of the chromophilic material.

Fig. 7.—Portion of the distal end of the nebenkern showing the constriction of the chromophobic material to form the tail vesicles.

Fig. 9.—Cross-sections of the nebenkern at the stage of fig. 8: *A* and *B*, through the region of the chromophilic material; *C*, through the chromophobic material above the plane of *A* and *B*.

Fig. 11.—Frequent appearance of tail vesicles when fully formed.

Fig. 12.—Cross-sections of the nebenkern in a condition slightly younger than that of fig. 10; *A*, through the chromophilic material; *B*, above (or below) the chromophilic material. From adjacent sections of the same nebenkern.

Fig. 14.—Small portion of a nebenkern showing the last remnant of chromophilic material just prior to its complete dissolution.

Plate 25.

Explanation of Figures.

All the figures are from Pygaera bucephala. Figs. 28, 29, and 30 are from the same cyst.

Figs. 15–41.—Progressive stages in the construction and fixation of the acrosome. The material of the acrosomal granule is stained intensely black in every case. The acrosomal vesicle can be clearly differentiated in figs. 15 to 19 and fig. 21. In fig. 34 are shown cross-sections of the acrosome at the stage (approximately) of figs. 32 and 33. The total length of the acrosome in the later stages of its elongation is approximated as nearly as conditions will permit. As a rule it could not be exactly determined owing to the extreme tenuity of the tip.

Plate 26.

Explanation of Figures.

All the figures are from Callosamia promethea. Fig. 44 is from the small generation of spermatids.

Fig. 42.—Primary spermatocyte, growth period, to show the chondriosomes.

Fig. 43.—Final telophase of the second spermatocyte division, showing first step in the condensation of the nebenkern.

Figs. 44, 46, 48, and 49.—Progressive stages in the transformation of the nebenkern and the deposition of the acrosome. The acrosome (in figs. 46, 48, and 49) appears as a more or less darkly-stained spherical body in contact with the nuclear membrane, and closely related to adjacent Golgi bodies.

Fig. 45.—Cross-section of the nebenkern in an early stage of elongation.

Fig. 47.—Cross-sections of the nebenkern at the stage of fig. 46: *A*, through the region of the chromophilic material; *B*, immediately above or below the chromophilic material; *C*, near the much elongated, free (or attached) end of the nebenkern.

Figs. 50–60.—Progressive stages in the formation of the definitive acrosome. The length of the acrosome has been approximated as closely as possible in figs. 58 and 59. In fig. 60 only a part of the acrosome is shown.